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Quick Reference Card

Muse® EGFR-RTK Activation Dual Detection Kit

MCH200102

To measure the extent of EGFR phosphorylation relative to the total EGFR expression in any given cell population

For Research Use Only. Not for use in diagnostic procedures.

Storage Conditions

All reagents must be stored at 2 - 8ºC.

Kit Components

- 20X Anti-phospho-EGFR (Tyr1173), Alexa Fluor® 555: (Part No. CS208204), 250 uL
- 20X Anti-EGFR, PECy5: (Part No. CS208205), 300 uL
- 5X Assay Buffer: (Part No. CS202124), 55 mL
- Fixation Buffer: (Part No. CS202122), 13 mL
- Permeabilization Buffer: (Part No. CS202125), 13 mL

Materials Recommended

- Guava® Muse® Cell Analyzer
- Test tubes for sample preparation and storage
- Tissue culture reagents, i.e. HBSS, PBS w/o Ca^{2+} or Mg^{2+}, cell dislodging buffers, etc.
- Micropipettes and tips
- Deionized water
- Cells of interest in suspension
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalogue No. 16466-030, or equivalent)

Assay Protocol

1. Prepare cell cultures for experimentation (treated or untreated).
2. Centrifuge cells at 300 x g for 5 min and wash once with 1X PBS.
3. Fix cells in Fixation Buffer for 5 min on ice, followed by a washing step
4. Permeabilize cells with Permeabilization Buffer for 5 min on ice, followed by a washing step
5. Add 200,000 cells to each tube (treated or untreated)
6. Add 10 µL of the antibody cocktail to 90 µL of 1X Assay Buffer per tube/test. Allow to incubate for 30 min at room temp (dark)
7. Centrifuge cells at 300 x g for 5 minutes and wash with 1X Assay Buffer
8. Resuspend in 200 µL 1X Assay Buffer
9. Acquire samples on Guava® Muse® Cell Analyzer.

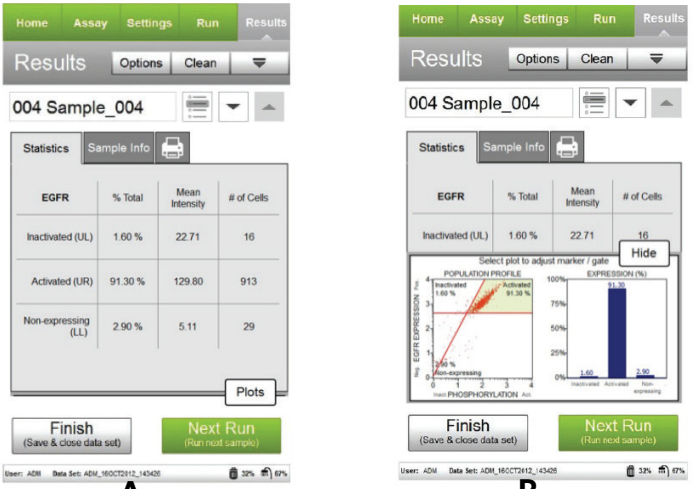
Expected Results

A431 cells were exposed to 100 ng/mL EGF for 5 minutes to induce an EGFR signaling cascade response, fixed, permeabilized, and then stained with both anti-phospho-EGFR (Tyr1173) and anti-EGFR antibodies in multiplex. Samples were acquired using the Guava® Muse® Cell Analyzer and statistical results are shown. Figure A shows the results summary, while Figure B shows results displayed by both dot plot and bar graph data.

The statistics captured in this assay show the relative percentages for each population as it is calculated within the total cell population. Cells which express EGFR can be seen by the data on the top two quadrants of the dot plot (inactivated and activated), representing about 93% of the total cell population. But of this cell population, 91% is activated upon treatment, indicating activation of the EGFR signaling pathway is present. By presentation of both datasets, one can now determine the total: phospho ratio within their testing samples.

For more information, please refer to the comprehensive EGFR-RTK Activation Dual Detection User's Guide for this product, which can be found at: *www.luminexcorp.com/flowkits*.

Figures A and B



Figures A and B show an example of results obtained using the Muse EGFR-RTK Activation Dual Detection Kit

Related Products

Description	Catalogue No.
Muse® Bcl-2 Activation Dual Detection Kit	MCH200105
Muse® H2A.X Activation Dual Detection Kit	MCH200101
Muse® MAPK Activation Dual Detection Kit	MCH200104
Muse® PI3K Activation Dual Detection Kit	MCH200103
Muse® System Check Kit	MCH100101
Muse® Count & Viability Kit (100T)	MCH100102

The latest version of Muse software, which includes all assay modules, as well as the kit user's guide, can be found at: *www.luminexcorp.com/flowkits*.

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